

Decreased expression of the *APOA1–APOC3–APOA4* gene cluster is associated with risk of Alzheimer's disease

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Background: Apolipoprotein is genetically associated with the risk of Alzheimer's disease (AD). The *APOA1*, *APOC3*, and *APOA4* genes are closely linked and located on human chromosome 11. Therefore, this gene cluster may be related to the risk of AD.

Patients and methods: A total of 147 AD patients and 160 healthy controls were randomly recruited from June 2013 to August 2014. *APOA1*, *APOC3*, and *APOA4* levels were measured using real-time quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay.

Results: *APOA1*, *APOC3* and *APOA4* levels were significantly lower in AD patients than controls ($P < 0.01$). *APOA1*, *APOC3*, and *APOA4* levels were negatively related with the severities of AD determined by Clinical Dementia Rating scores ($P < 0.01$). *APOA1*, *APOC3*, and *APOA4* levels showed a negative relation with Montgomery–Åsberg Depression Rating Scale scores and a positive relation with RAND 36-item health-survey scores ($P < 0.01$). There was a decreased trend for levels of *APOA1*, *APOC3*, and *APOA4* in AD patients.

Conclusion: Low levels of *APOA1*, *APOC3*, and *APOA4* are associated with risk of AD. *APOA1*, *APOC3*, and *APOA4* should be developed as combined drugs for the therapy of AD.

Keywords: Alzheimer's disease, *APOA1–APOC3–APOA4* gene cluster, enzyme-linked immunosorbent assay, Montgomery–Åsberg Depression Rating Scale, RAND 36-item health survey, real-time quantitative reverse-transcriptase PCR

Introduction

Alzheimer's disease (AD) is a leading cause of dementia among the elderly and affects over 24 million people worldwide, and this number could increase twofold within two decades.¹ The number of AD patients is increasing with the aging of the world population. AD is mainly characterized by amyloid plaques that are often surrounded by neurofibrillary tangles in the brain and poor memory.^{2,3} However, the etiology of AD is complex, and more molecular mechanisms still need to be explored.

To define ideal therapy strategies, early diagnosis and early intervention is crucial for timely therapy of AD. Although the neuropathology of AD can be found by autopsy, confirming prognostic characteristics of AD patients is still far from straightforward.⁴ Cerebrospinal fluid (CSF) biomarkers, such as total tau, phosphorylated tau, and $A\beta_{42}$, have high accuracy during the early diagnosis of AD patients.⁵ Current AD guidelines give these criteria for the diagnosis of AD patients according to increased levels of phosphorylated tau, total tau, and $A\beta_{42}$ in CSF.⁶ However, a spinal tap is used to obtain CSF.⁷ On the other hand, taking biopsies is often invasive and extremely difficult to perform. Not all AD patients want to accept the diagnosis; therefore, new methods are much needed.

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Potential biomarkers for AD patients have been widely reported. Theoretically, DNA damage should be associated with the development of AD. Biomarkers for DNA damage have been reported in AD patients.⁸ Many serum biomarkers for AD patients have been also reported, such as IGF-1. IGF-1 enters mammalian brains and promotes amyloid peptide clearing, which prevents amyloid peptide accumulation in the brain. Decreased IGF-1 levels have been observed in AD patients.⁹ Also, ADNP levels are reduced in the development of AD.¹⁰ ADNP maintains cell survival by regulating the levels of p53, which protects cerebral cortical neurons by inhibiting the aggregation of A β ₄₂ in the brain, and thus ADNP plays an important role in inhibiting the development of AD.¹¹ Recent advances in AD biomarkers have inspired novel research criteria that reconceptualize the diagnosis of AD involving two aspects: cognitive variation and structural evidence of AD pathogenesis.¹²

APOE is a kind of apolipoprotein in the chylomicron and an intermediate-density lipoprotein, which is necessary for the normal catabolism of lipoprotein with rich triglyceride (TG). In the central nervous system, APOE is produced via astrocytes, and can transport cholesterol to neurons.¹³ The APOE allele has been reported to be related to the risk of AD.^{14,15} APOA4, a component of lipoprotein particles similar to APOE, has been suggested to play an important role in brain metabolism. APOA4 deficiency can increase the levels of A β deposition in the brain and result in cognitive damage in an animal model of AD.¹⁶ APOA4 polymorphism has been reported as a risk factor for an unfavorable lipid serum profile.¹⁷ On the other hand, a growing body of evidence suggests that obesity, hypertriglyceridemia, and reduced high-density lipoprotein cholesterol (HDL-c) is important in the development of cognitive impairment and AD. APOA1 was also observed at lower levels in AD patients via proteomic two-dimensional gel electrophoresis in 2006.¹⁸ A clinical study has shown low HDL-c and APOA1 levels in patients with AD.¹⁹ APOC3 polymorphism has been reported to be associated with AD in a Chinese population.²⁰ Furthermore, the *APOA1*, *APOC3*, and *APOA4* genes are closely linked on the long arm of human chromosome 11.²¹ The *APOA1* and *APOA4* genes are located on the same strand, while the *APOA1* and *APOC3* genes are convergently transcribed. Increased APOC3 levels induce the progression of hypertriglyceridemia.²² On the other hand, the gene polymorphism has been widely reported to be associated with AD related to cerebral lipoprotein homeostasis, including APOA1, APOC3, and APOA4.²³ Therefore, all these genes may have synergistic functions, and their low-level expression may be associated with the risk of AD.

Patients and methods

Participants

All the protocols were approved by the institutional ethical committee of China Medical University and conducted in accordance with the Declaration of Helsinki.²⁴ All subjects were Han Chinese from Shenyang. Informed consent was obtained from each subject directly or from his or her guardian. From June 2013 to August 2014, a total of 147 patients were recruited at the Fourth Affiliated Hospital of China Medical University (Shenyang). All AD patients were diagnosed with National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria according to a previous report.²⁵ All familial cases of AD were excluded in this study. Patients were excluded if they had a history of suicidal behavior, severe substance abuse, difficulty with verbal conversation, and brain injuries or other brain disorders.

Study design

After all AD patients had been screened, 147 patients were eligible for the experiment. Meanwhile, 160 healthy subjects were selected as a control group. The two groups were similar in terms of sex ratio, age, body mass index (BMI), educational levels, alcohol consumption, and cigarette smoking.

Neuropsychological tests

All cases were diagnosed by licensed neurological physicians according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM): neurological examination, blood tests, and neuroimaging data (computed tomography or magnetic resonance imaging). The Clinical Dementia Rating (CDR) scale is frequently used to detect memory impairment and quantify the severity of dementia.²⁶ The aim of the clinical diagnosis for some dementia (as a frontal-temporal dementia) is not memory impairment in the acute phase.²⁷ The CDR is used to assess three domains of cognition (memory, orientation, problem solving) and three domains of function (community affairs, home and hobbies, personal care).²⁸ The Mini Mental State Examination (MMSE) is used to diagnose dementia, assess its progression and severity, and examine memory problems.

All participants were evaluated with the Kimberley Indigenous Cognitive Assessment of Depression based on the World Health Organization *International Classification of Diseases* and the DSM-IV-TR of the American Psychiatric Association.²⁹ There are eleven items in the Kimberley Indigenous Cognitive Assessment of Depression, each of which is assigned from "never" to "sometimes", "a lot", and "all the time" based on a frequency scale.

There are numerous factors causing AD. In order to reduce the interference of these factors, age, sex, years of education, and career were compared between a control group and an experimental group.^{30,31} Furthermore, to exclude the effects of compound factors, all subjects were selected from Shenyang and lived in families with similar educational and economic background. Depression is a frequent condition in AD patients, so depression was also considered here. The severity of depression was examined based on the Montgomery–Åsberg Depression Rating Scale (MADRS).³² There are ten items in the MADRS, scored from 0 to 60. Severity of depression is graded as severe, moderate, and mild based on cutoff scores.³³ Psychopathological dysfunction in emotion is often described using anorexia, which is also related with depression.

Anxiety is often associated with AD and measured based on the standard of the Hospital Anxiety and Depression Scale.^{34–36} Hospital Anxiety and Depression Scale anxiety scores are presented from 0 to 21, and higher scores stand for severe anxiety. Alexithymia was investigated based on the Toronto Alexithymia Scale.³⁷ General functioning was tested by the Global Assessment of Functioning scale.³⁸ Life quality was evaluated using the RAND 36-item health-survey (RAND-36), which assesses well-being and functioning in eight dimensions.³⁹

mRNA extraction

Venous blood (5 mL) was taken from every participant after obtaining signed consent forms. Venous blood was centrifuged at 3,000 *g* for 10 minutes and the serum collected. Total RNA was isolated from serum by an RNeasy Mini Kit (Qiagen NV, Venlo, the Netherlands).

Real-time qRT-PCR

Messenger RNA (mRNA; 1 μ L) was reverse-transcribed to complementary DNA by reverse transcription using AMV Reverse Transcriptase (Promega Corporation, Fitchburg, WI, USA). The gene fragments were further amplified using quantitative real-time polymerase chain reaction (qRT-PCR) with primers for *APOA1* (forward primer 5'-gtgetcaaagacagcgccag-3', reverse primer 5'-ctcctctcctgcctcagcc-3', 200 bp), *APOC3* (forward primer 5'-tcagaggccgaggatgcctc-3', reverse primer 5'-cttgcttaacggtgctcc-3', 180 bp), *APOA4* (forward primer 5'-ctcttcagacaacttg-3', reverse primer 5'-tccagctccttccaatctc-3', 140 bp) and *GAPDH* (forward primer 5'-gatccctccaaaatcaagt-3', reverse primer 5'-atactctcatggtccacac-3', 180 bp). PCR was conducted

with a LightCycler FastStart DNA Master SYBR Green I (Hoffman-La Roche Ltd., Basel, Switzerland) on an ABI 7500 real-time PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA). The PCR was performed as follows: 45 cycles (denaturation, 5 seconds at 95°C; annealing, 5 seconds at 60°C; extension, 20 seconds at 72°C).

ELISA analysis for protein levels of APOA1, APOC3, and APOA4

The protein levels of APOA1, APOC3, and APOA4 were measured in serum using a Human APOA1 ELISA (enzyme-linked immunosorbent assay) Kit (ab108804; Abcam, Cambridge, MA, USA), a Human APOC3 ELISA Kit (ab154131; Abcam), and a Human APOA4 ELISA Kit (P06727; Beacon Analytical Systems Inc, Saco, ME, USA). To determine serum levels of APOA1, APOC3, and APOA4, 5 mL blood was drawn into a vacuum tube. The blood was centrifuged at 3,000 *g* for 10 minutes, and the serum was stored at -80°C. Serum APOA1, APOC3, and APOA4 were measured using ELISA kits.

Biochemical enzyme analysis

APOA1 may reduce the oxidative stress in mammals, considering APOA1 can increase concentrations of HDL, which has potential antioxidative activities.⁴⁰ SOD, AST, ALT and GSH have been regarded as biomarkers for oxidative stress.^{41–46} Therefore, the levels of all enzymes were examined using the blood samples of participants. AST and ALT levels were measured with a biochemical analysis instrument (Beckman Coulter Inc, Pasadena, CA, USA). SOD activity was analyzed using a previously reported method.⁴⁷ GSH levels were determined using a fluorometric method.⁴⁸

Analysis of lipid profiles

APOA1, APOC3, and APOA4 are key molecules for modulating lipid metabolism, which is associated with changes in TG, total cholesterol (TC), HDL-c, and low-density lipoprotein cholesterol (LDL-c) concentrations. Therefore, TG, TC, HDL-c and LDL-c levels were also measured in blood samples using a biochemical analyzer. Malondialdehyde, a biomarker of oxidative damage, was analyzed according to a previously reported method.⁴⁹

Statistical analyses

All statistical analyses were performed with SPSS 20.0 (IBM Corporation, Armonk, NY, USA). AD patients and controls were compared using *t*-tests. One-way analysis of variance was performed to explore the association of APOA1, APOC3,

and APOA4 levels and AD patients. There was a statistically significant difference if $P < 0.05$. Spearman's rank correlation coefficient was used to investigate the relationship between the degrees of depression (MADRS or RAND-36) and the protein levels of APOA1, APOC3, and APOA4. The sample size of the association was measured using Quanto 1.2. The level of significance was set at $P = 0.05$, and the confidence interval (CI) set at 95%.

Results

Baseline characteristics of participants

The a priori sample size for the AD study was estimated with a study power of 90% to have a decrease in serum APOA1, APOC3, and APOA4 levels in AD patients compared with the controls, assuming an α -level of 0.05. The present study was initiated because of previous concerns over AD patients enrolled in our hospital. Finally, the number of AD patients was more than our expectations, and a priori size was determined and attained in the study.

Table 1 shows baseline characteristics of all participants. A total of 147 AD patients received medical and neurological test (male/female 63/84, mean age at study 68.9 years, range

57.1–80.7 years) (Table 1). All 160 controls were diagnosed as cognitively intact after receiving medical and neurological tests (male/female 66/94, mean age at study 69.6 years, range 57.1–82.1 years).

Controls matched AD patients well with regard to sex and age ($P > 0.05$). According to previous reports, BMI is related to the development of AD.^{50,51} To avoid the interference of BMI, the controls were carefully chosen to make sure that there was no significant statistically difference between AD patients and controls ($P > 0.05$) (Table 1). Therefore, BMI was not a risk factor for AD patients. Diabetes affects many elderly people worldwide, with a deep decrease in life quality. Meanwhile, blood pressure and heart disorders are also risk factors of AD.^{52,53} On the other hand, smoking is related to a decrease in gray-matter density in brains, which contributes to the onset of AD.⁵⁴ Drinking alcohol impairs brain function and causes dementia and geriatric cognitive disorders.^{55,56} Individuals with long-term education will have better cognitive function than those with short-term education.^{30,57} During the recruiting period, all the parameters were also carefully measured to avoid heterogeneous effects on final results (Table 1).

Table 1 Baseline characteristics of AD patients and healthy controls

	AD patients	Control (n=160)	t-value/ χ^2 statistic	P-value
Age, years, mean (SD)	68.9 (11.8)	69.6 (12.5)	0.145	0.892 ^a
Male, n (%)	63 (42.9)	66 (41.3)	0.081	0.776 ^b
Smoker/nonsmoker	72/75	78/82	0.158	0.691 ^b
Drinker/nondrinker	73/74	79/81	0.003	0.960 ^b
Education level (>9 years), n (%)	32 (21.8)	34 (21.3)	0.012	0.912 ^b
Spouse, n (%)	132 (89.8)	136 (85.0)	1.589	0.207 ^b
Career (working overtime), n (%)	93 (63.3)	98 (61.3)	0.132	0.716 ^b
Anxiety (cutoff score 8 in Hospital Anxiety and Depression Scale), n (%)	44 (29.9)	5 (3.1)	41.044	0.000 ^b
Current medication, n (%)				
Antidepressant medication	51 (34.7)	0	152.031	0.000 ^b
Selective serotonin-reuptake inhibitors	63 (42.9)	0	86.276	0.322 ^b
Serotonin–noradrenaline-reuptake inhibitors	21 (14.3)	0	24.535	0.000 ^b
Psychiatric test scores, mean (SD)				
Montgomery–Åsberg Depression Rating Scale	23.9 (7.8)	14.2 (2.4)	2.141	0.004 ^a
Anxiety score (Hospital Anxiety and Depression Scale)	12.6 (4.5)	4.3 (1.1)	2.649	0.002 ^a
Global Assessment of Functioning score	59.4 (6.8)	78.1 (8.3)	3.517	0.001 ^a
Toronto Alexithymia Scale – 20	54.6 (13.8)	23.2 (6.3)	4.388	0.001 ^a
RAND-36 score	52.3 (16.1)	83.6 (10.9)	3.046	0.002 ^a
MMSE scores	19.8±4.9	31.8±4.3	3.061	0.002 ^a
CDR scale				
CDR 0, n	0	160	307.021	0.000 ^b
CDR 0.5, n	84	0	125.868	0.000 ^b
CDR 1, n	36	0	44.388	0.000 ^b
CDR 2+, n	27	0	32.221	0.000 ^b

Notes: ^aStudent's *t*-test; ^bstandard χ^2 statistic.

Abbreviations: AD, Alzheimer's disease; SD, standard deviation; MMSE, Mini-Mental State Examination; CDR, Clinical Dementia Rating.

Neuropsychological tests showed that all AD subjects had MMSE scores less than 24.7, while all controls had MMSE scores more than 27. AD patients had an overall CDR score of 0.5 or more than 0.5, while overall CDR scores of all controls were 0 (Table 1). These results suggested that AD patients had clinical cognitive diseases, while controls were free of cognitive disorders.

mRNA levels of serum APOA1, APOC3, and APOA4

The qRT-PCR showed that mRNA serum APOA1, APOC3, and APOA4 levels were lower in AD patients than controls. mRNA serum APOA1, APOC3 and APOA4 levels decreased when CDR scores increased from 0.5 to 2+ in AD patients. mRNA levels of APOA1, APOC3, and APOA4 were the lowest in AD patients with CDR 2+ ($P<0.05$) (Figure 1). Serum mRNA APOA1, APOC3, and APOA4 levels were negatively related with the development of AD ($P<0.01$).

ELISA analysis

The concentrations of APOA1, APOC3, and APOA4 in serum were determined by responding standard curves (Figure 2A, C, and E). APOA1 concentrations were 140 ± 40 ng/mL (95% CI 125–173 ng/mL) for healthy participants with CDR 0, 78 ± 16 ng/mL (95% CI 70–90 ng/mL) for AD patients with CDR 0.5, 55 ± 15 ng/mL (95% CI 50–65 ng/mL) for AD patients with CDR 1, and 35 ± 15 ng/mL (95% CI 28–42 ng/mL) for AD patients with CDR 2+ (Figure 2B). APOC3 concentrations were 395 ± 95 ng/mL (95% CI 320–440 ng/mL) for healthy participants with CDR 0, 180 ± 60 ng/mL (95% CI 130–210 ng/mL) for AD patients

with CDR 0.5, 80 ± 40 ng/mL (95% CI 60–100 ng/mL) for AD patients with CDR 1, and 38 ± 23 ng/mL (95% CI 30–50 ng/mL) for AD patients with CDR 2+ (Figure 2D). APOA4 concentrations were 595 ± 145 ng/mL (95% CI 475–690 ng/mL) for healthy participants with CDR 0, 220 ± 80 ng/mL (95% CI 170–240 ng/mL) for AD patients with CDR 0.5, 90 ± 50 ng/mL (95% CI 50–100 ng/mL) for AD patients with CDR 1, and 45 ± 25 ng/mL (95% CI 35–55 ng/mL) for AD patients with CDR 2+ (Figure 2F). Serum APOA1, APOC3, and APOA4 levels were also negatively associated with the progression of AD ($P<0.01$).

Oxidative stress in AD pathogenesis

Next, we explored whether oxidative stress plays an important role in AD pathogenesis. We measured SOD, AST, ALT, and GSH levels, which are biomarkers of oxidative stress.^{41–46} As Table 2 shows, SOD and GSH levels were lower in AD patients than those in controls. In contrast, AST and ALT levels were higher in AD patients than those from controls (Table 2). The results suggested that oxidative stress is involved in AD pathogenesis.

Serum lipid levels in AD patients

Biochemical analysis showed that serum TC, TG, LDL-c and malondialdehyde levels were higher in AD patients than controls ($P<0.05$) (Table 3). In contrast, serum HDL-c levels were lower in AD patients than controls ($P<0.05$) (Table 3). The results suggested that lipid peroxidation product was increased and antioxidant product was decreased in AD patients, which contributes to the development of AD. Elevated TC, LDL, and TG and decreased HDL characterized the lipid profile in AD, which is different from a previous report.⁵⁸ Further work is needed to validate the lipid profile as a biomarker for the development of AD.

Association between APOA1, APOC3, and APOA4 levels and MADRS scores

When severity of AD was stratified by MADRS, significant differences in APOA1, APOC3 and APOA4 levels were observed in different scores of MADRS. APOA1, APOC3, and APOA4 levels were reduced when MADRS scores increased in AD patients and controls (Figures 3A and C, 4A and C) ($P<0.001$). APOA1, APOC3, and APOA4 levels were negatively related with MADRS scores, suggested that APOA1, APOC3, and APOA4 inhibited the development of AD, since MADRS scores are often used to evaluate the severity of AD symptoms.⁵⁹

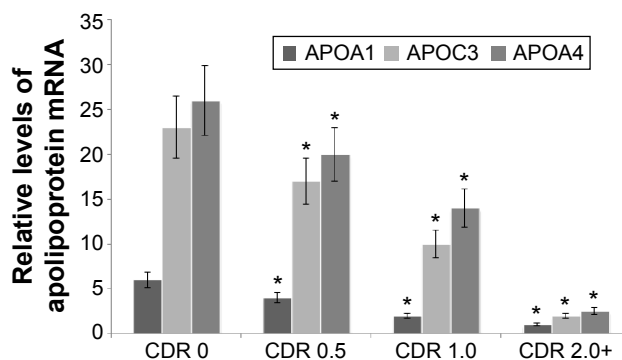


Figure 1 Quantitative RT-PCR analysis for APOA1, APOC3, and APOA4 messenger RNA (mRNA) levels in AD patients and controls.

Notes: Quantitative RT-PCR showed higher levels of APOA1, APOC3, and APOA4 mRNA from controls than those from AD patients. Each bar represents the mean \pm SD of five independent cases. * $P<0.05$ via CDR 0.

Abbreviations: RT-PCR, reverse-transcriptase polymerase chain reaction; AD, Alzheimer's disease; SD, standard deviation; CDR, Clinical Dementia Rating.

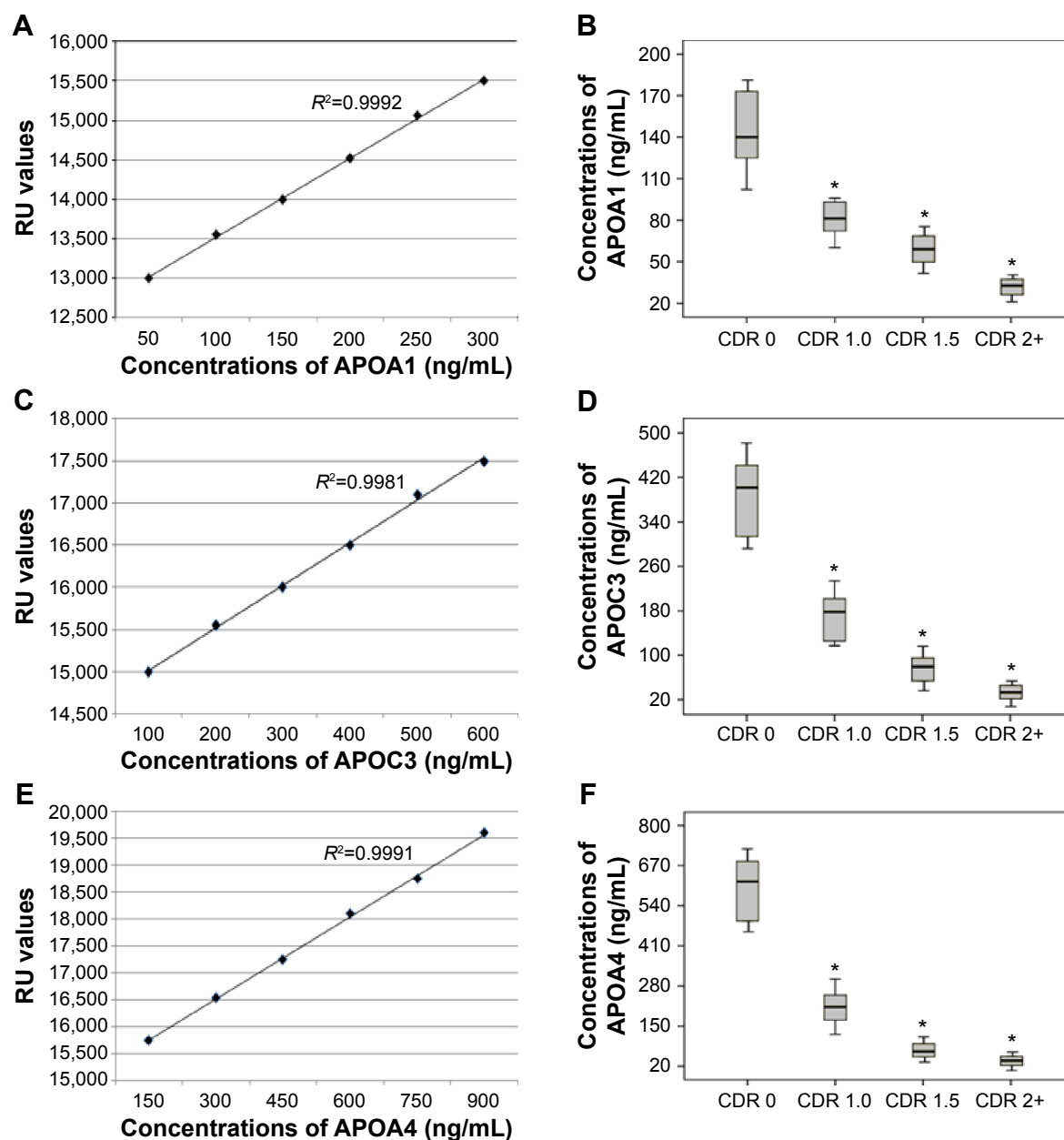


Figure 2 Measurement of serum APOA1, APOC3, and APOA4 by ELISA.

Notes: (A) A standard curve was made by measuring the concentration of APOA1 and absorbing values at 450 nm. (B) Bar diagram indicating the differences in serum APOA1 among all participants with different CDR degrees. (C) A standard curve was made by measuring the concentration of APOC3 and absorbing values at 450 nm. (D) Bar diagram indicating the differences in serum APOC3 among all participants with different CDR degrees. (E) A standard curve was made by measuring the concentration of APOA1 and absorbing values at 450 nm. (F) Bar diagram indicating the differences in serum APOA1 among all participants with different CDR degrees. The CDR score of healthy controls was 0 (n=160 cases), and the CDR scores of AD patients were 0.5 (n=73 cases), 1.0 (n=44 cases), and 2.0+ (n=30 cases). * $P < 0.05$ via CDR 0.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; AD, Alzheimer's disease; CDR, Clinical Dementia Rating; RU, repopulating unit.

Table 2 Biochemical parameters of enzyme activities

Group	SOD (U/mL)	GSH (ng/L)	ALT (U/mL)	AST (U/mL)
AD	11.252±4.361	12.223±1.973	89.791±6.485	212.247±19.698
Controls	27.144±3.082	26.258±2.567	47.228±10.83	112.554±24.077
t-value	5.482	6.948	7.125	5.916
P-value	0.002	0.001	0.001	0.002

Notes: All data presented as mean \pm SD (n=147 for AD patients and n=160 for healthy controls). Student's t-test used to evaluate statistically significant differences ($P < 0.05$).

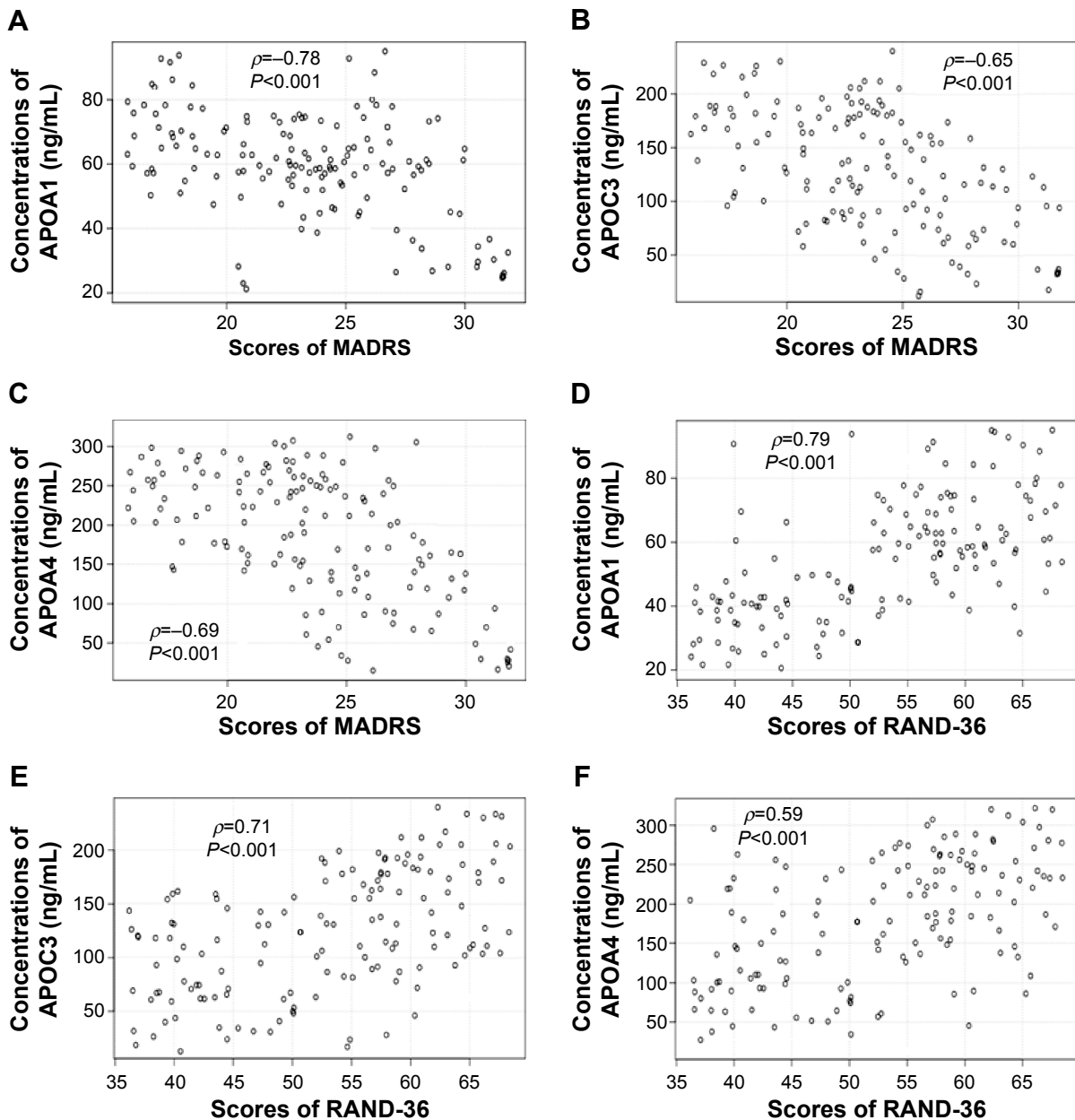
Abbreviations: GSH, glutathione; AD, Alzheimer's disease; SD, standard deviation; SOD, superoxide dismutase; ALT, alamine aminotransferase; AST, asparate aminotransferase.

Table 3 Biochemical parameters of lipid components

Group	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	MDA (nmol/L)
AD	1.29±0.42	3.59±0.82	0.97±0.49	2.85±0.76	1.43±0.41
Controls	0.83±1.17	2.56±0.88	1.35±0.59	1.93±0.48	0.86±0.14
t-value	1.589	1.884	2.241	3.589	2.547
P-value	0.045	0.040	0.024	0.016	0.036

Notes: All data presented as mean ± SD (n=147 for AD patients and n=160 for healthy controls). Student's t-test was used to evaluate statistically significant differences ($P<0.05$).

Abbreviations: TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde; AD, Alzheimer's disease; SD, standard deviation.

**Figure 3** Relationship between MADRS or RAND-36 scores and APOA1/APOC3/APOA4 levels in AD patients.

Notes: (A) Relationship between MADRS scores and APOA1 levels. (B) Relationship between MADRS scores and APOC3 levels. (C) Relationship between MADRS scores and POA4 levels. (D) Relationship between RAND-36 scores and APOA1 levels. (E) Relationship between RAND-36 scores and APOC3 levels. (F) Relationship between RAND-36 scores and APOA4 levels. Statistical analysis performed using Spearman's rank correlation test. There is a strong negative correlation if ρ -values are between -1 and -0.5 , and there is a strong positive correlation if ρ -values are between 0.5 and 1 .

Abbreviations: MADRS, Montgomery–Åsberg Depression Rating Scale; AD, Alzheimer's disease.

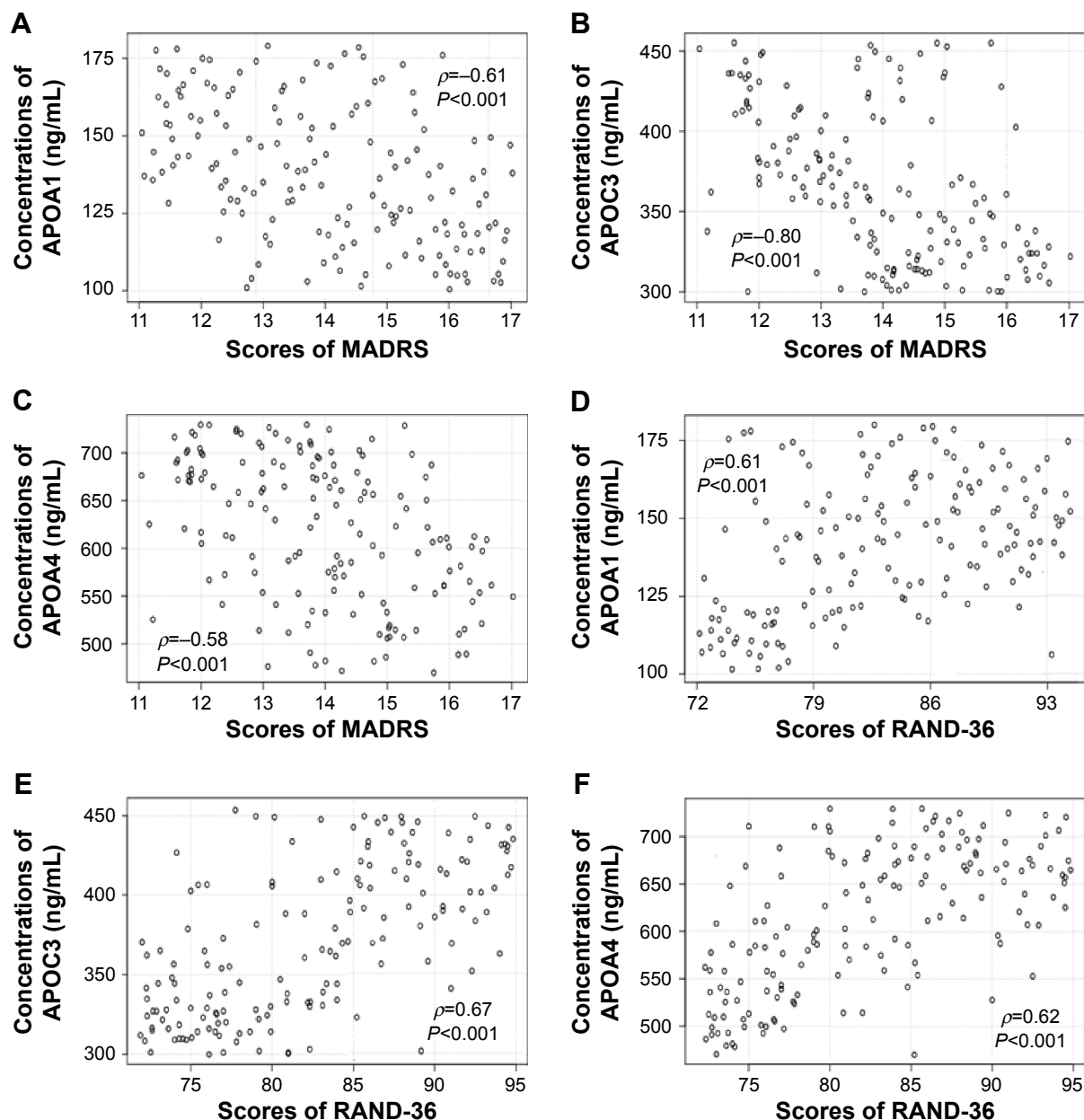


Figure 4 Relationship between MADRS or RAND-36 scores and APOA1/APOC3/APOA4 levels in healthy subjects.

Notes: (A) Relationship between MADRS scores and APOA1 levels. (B) Relationship between MADRS scores and APOC3 levels. (C) Relationship between MADRS scores and APOA4 levels. (D) Relationship between RAND-36 scores and APOA1 levels. (E) Relationship between RAND-36 scores and APOC3 levels. (F) Relationship between RAND-36 scores and APOA4 levels. Statistical analysis was performed using Spearman's rank correlation test. There is a strong negative correlation if ρ -values are between -1 and -0.5 . There is a strong positive correlation if the ρ -values are between 0.5 and 1 .

Abbreviation: MADRS, Montgomery-Åsberg Depression Rating Scale.

Association between APOA1, APOC3, and APOA4 levels and RAND-36 scores

RAND-36 is a reliable, valid, and sensitive method for measuring general health.⁶⁰ When the severity of AD was stratified by RAND-36, different APOA1, APOC3, and APOA4 levels were observed with different RAND-36 scores. APOA1, APOC3, and APOA4 levels increased when RAND-36 scores increased (Figures 3D and F, 4D and F) ($P < 0.001$). APOA1, APOC3, and APOA4 levels were

positively related with MADRS scores, suggesting that APOA1, APOC3, and APOA4 inhibit the development of AD, since RAND-36 score is often used to evaluate general health.

Discussion

The high incidence and financial burden of AD greatly reduces the life quality of human beings. Although pharmaceutical therapy has been accepted as the first choice for

AD patients, serious adverse effects and high-cost medical therapies have limited the utilization of the medicine. A new concept is also needed to understand the mechanisms by studying symptoms, behaviors, or biomarkers, which are different to traditional classification for mental disorders.⁶¹ Meanwhile, the study can also provide valuable information for the therapy of widespread depression.

Early detection of AD is also a great challenge for the therapy of dementia, and more molecular mechanisms urgently need to be explored. A combination of diet, lifestyle, and vascular and genetic elements that promote the onset and course of AD may be more likely the cause of AD. The possibility that AD risk can be reduced by diet is of great importance, and suggests a nonpharmaceutical therapy for AD. Because of the importance of lipid diets and metabolism in preventive treatment against AD, lipid metabolism may play an important role in AD risk.⁶² AD patients have significantly higher TC and LDL than patients with other dementia, which has been confirmed in a larger cohort. The result showed that increasing certainty of AD was closely associated with higher-level TC and LDL.⁶³ In contrast, a conflict finding has been also reported: lipid profile is connected with the etiology and progress of AD. There was an association between low serum cholesterol and LDL-c levels and cognitive decline in patients with AD.⁵⁸

Presently, the molecular mechanisms linking lipid and AD pathology remain unclear. No data are available on serum apolipoprotein in AD patients yet. Furthermore, the cause of AD is much more complex and not only a single genetic cause. Three genes – *APOA1*, *APOC3*, and *APOA4* in a gene cluster – may have synergetic enhancement effects on a healthy lipid profile and are often closer to one another. Therefore, we examined the association between the expression levels of the three genes and AD risk. The results showed that low serum levels of three apolipoproteins were correlated with development of AD based on CDR scores. Apolipoprotein concentrations declined significantly in AD patients, but not in controls. Therefore, decreased expression of serum *APOA1*, *APOC3*, and *APOA4* may be related with risk of AD.

Nevertheless, one important question should be clarified. The AD pathological process occurs in brain. How can *APOA1–APOC3–APOA4* levels in serum reflect AD pathogenesis? Serum lipid may be independent of the brain, but an unfavorable lipid profile may cause hyperlipidemia and subtle changes in brain lipid metabolism.⁶⁴ Also, Yu et al found that a long-term high-lard diet improved serum TC and LDL-c levels, which also could affect the brain's lipid composition, memory, and learning activities.⁶⁵ Here, we considered more

that serum apolipoproteins can be measured directly and will be useful as indicators to evaluate AD risk.

Compared with other serum biomarkers of AD patients,^{9,10} serum apolipoproteins *APOA1*, *APOC3*, and *APOA4* seem to be more functional proteins: 1) three proteins can be used to predict the severity of AD more accurately than a single protein, 2) all the proteins affect the progression of AD,^{66,67} and 3) the polymorphisms of these apolipoprotein genes may result in the pathogenesis and development of AD.^{20,68} With more certain results, we hope that apolipoprotein-gene cluster of *APOA1*, *APOC3*, and *APOA4* can be widely applied in the diagnosis of AD.

Certainly, there are some limitations in the present work. Firstly, the present trial had its limitations in a statistical sense and was not conducted in a larger population. More work is needed to be performed in a larger population to validate the present results. Secondly, a gene cluster still cannot present a complete study of AD. The molecular mechanism for the effects of *APOA1*, *APOC3*, and *APOA4* on the progression of AD remains unknown. A series of silencing and overexpression studies are needed to make sure of their functional role in the risk of AD. Thirdly, the polymorphism of these apolipoprotein genes will result in the pathogenesis and development of AD, which was not considered here. Nonetheless, the present work will be beneficial for understanding molecular mechanisms for the risks of AD.

In sum, the results of this work suggest that decreased expression of the *APOA1–APOC3–APOA4* gene cluster may be associated with AD risk. *APOA1*, *APOC3*, and *APOA4* can reduce the severity of AD symptoms by increasing RAND-36 scores and reducing MADRS scores. Therefore, *APOA1*, *APOC3*, and *APOA4* should be developed as new combined biomarkers for the early diagnosis of AD patients.

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Disclosure

The authors report no conflicts of interest in this work.

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